

# COMPARATIVE ANTIBACTERIAL ACTIVITY OF HONEY AND GENTAMICIN AGAINST *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA*

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**SUMMARY.** The clinical significance of *Pseudomonas aeruginosa* and *Escherichia coli* is a strong factor for regular monitoring of their sensitivity to both established and novel antimicrobial compounds. Human isolates of these organisms were collected from different pathological sources and tested for their sensitivity to gentamicin - an established aminoglycoside antibiotic - and to honey, a natural product that is generating renewed interest for its therapeutic application. In an agar-cup diffusion method, three undiluted different samples of honey and their 1:2 to 1:6 aq. dilutions showed activity on 100% and 96.4% respectively of *Pseudomonas aeruginosa* isolates compared with 95.4% of *Escherichia coli* using either of the undiluted or 1:2 aq. dilutions of the honey samples. Gentamicin used in concentrations of 8.0 and 4.0 µg/ml varied in its activity against both organisms but was generally lower than the antibacterial activity of each undiluted honey and its 1:2 aq. dilution. In the event of therapeutic failure with gentamicin or any other related antibiotics, honey offers a suitable and better alternative in managing infected burn wounds and other forms of infected wounds as well as prophylaxis in trauma wounds.

## Introduction

*Pseudomonas aeruginosa* owes its clinical significance to the fact that it is an aetiology of a good number of infections such as septic burns and wounds, conjunctivitis, endocarditis, meningitis, and urinary tract infections. Notably, it serves as a reference species in antimicrobial susceptibility testing on account of its notorious resistance to most antimicrobial compounds.<sup>1</sup>

Similarly, *Escherichia coli*, though normally a gut commensal, has attracted clinical significance owing to the recognition of several strains of diarrhoeagenic *E. coli* with distinct virulent factors. Collier et al.<sup>2</sup> identified these strains as enteropathogenic, enterotoxigenic, enteroinvasive, verocytotoxin-producing, enteroaggregative, and diffusively adherent *E. coli* (EPEC, ETEC, EIEC, VTEC, Egg EC, and DAEC, respectively).

Gentamicin is a standard antibiotic noted for its activity against Gram-negative bacteria, especially in combination with vancomycin or a penicillin.<sup>3</sup> At a concentration of 4.0 µg/ml, it exerted pronounced activity against *P. aeruginosa* 41 NCTC 6750 (unpublished work<sup>4</sup>). Similarly, honey has been associated with antibacterial and antifungal activity.<sup>5</sup> Specifically, *P. aeruginosa* and *E. coli* were among the three laboratory isolates that had their growth inhibited by honey.<sup>6</sup> Ibrahim<sup>7</sup> and Jeddar et al.<sup>8</sup> reported bactericidal activity of honey on *Salmonella* spp. and *Shigella* spp. as also enteropathogens such as *E. coli*, *Vibrio cholerae* and other Gram-negative and Gram-positive

bacteria.

Comparative studies have however identified honey as a more effective remedy than some antimicrobial compounds. This was the situation found between honey and silver sulphadiazine and between honey and certain antibiotics.<sup>9-12</sup> This study reports the antibacterial activity of honey from three different sources and of gentamicin on isolates of *P. aeruginosa* and *E. coli* from different pathological sources.

## Materials and methods

### Bacteriology

A total number of fifty isolates of *P. aeruginosa* and *E. coli* from various pathological sources (Table I) were collected on sterile nutrient agar (OXOID) slants from the Routine Section of the Medical Microbiology, Laboratory, University College Hospital, Ibadan, Nigeria. The pseudomonal isolates were purified on cetrimide agar and the escherichial isolates on eosin methylene blue agar. Both sets of isolates were confirmed by conventional tests<sup>12</sup> and then preserved on fresh nutrient agar slants in a refrigerator at 4 °C.

### Honey

Honey was obtained from three pure natural honey collection centres (A, B, and C) in Ibadan and Abeokuta, South West Nigeria. Each stock was used undiluted and also as fresh aq. dilutions of 1:2, 1:4, and 1:6 against the

**Table I** - Pathological sources of *P. aeruginosa* and *E. coli*

Pathological source	<i>P. aeruginosa</i> Number of isolates	<i>E. coli</i> Number of isolates
Pus	3	—
Wound swab	10	10
Ear swab	10	7
Wound biopsy	1	—
Burn wound	2	—
Sputum	1	—
Control strain (NCTC)	1	1
Throat swab	—	1
Urine	—	2
Tracheal swab	—	1
Total	28	22

respective bacterial isolates tested.

#### Gentamicin

Gentamicin sulphate (BP), a product of Medreich, India, was obtained in ampoule vials (2 ml) from a local pharmacy store. The antibiotic was used in 4 and 8 µg/ml (aq.) dilutions alongside honey against every bacterial isolate.

#### Sensitivity test

The agar-cup diffusion method<sup>6,13</sup> was employed to obtain the susceptibility pattern of the bacterial isolates against each undiluted honey and its fresh aq. dilutions and of 4 and 8 µg/ml of gentamicin. Considerations for the sensitivity and resistance of bacteria were based on the extent of the presence or absence of zones of growth inhibition.<sup>13</sup>

**Table II** - Sample results of sensitivity test on honey and gentamicin against *E. coli* and *P. aeruginosa*

<i>E. coli</i>															
Isolate no.	Source	Honey A				Honey B				Honey C				Gent. (µg/ml)**	
		0*	1:2 <sup>a</sup>	1:4	1:6	0	1:2	1:4	1:6	0	1:2	1:4	1:6	4:0	8.0
Control strain	NCTC UK	18.5 <sup>b</sup>	14.5	9.9	— <sup>c</sup>	17.9	12.5	—	—	15.5	11.9	—	—	12.5	15.9
17	Wound swab	25.5	15.9	12.5	—	23.9	15.5	10.9	—	19.9	—	—	—	10.9	14.9
15	Wound swab	22.5	14.5	13.9	10.5	18.5	15.5	11.5	—	19.9	—	—	—	—	9.5
<sup>126</sup> / <sub>108</sub>	Wound swab	—	—	—	—	—	—	—	—	—	—	—	—	10.9	13.5
106	Wound swab	10.9	8.9	—	—	9.4	6.5	—	—	6.9	4.5	—	—	—	—
48	Wound swab	12.9	10.9	—	—	8.5	—	—	—	6.9	—	—	—	—	—
279	Ear swab	12.5	7.5	5.9	—	8.9	5.5	4.9	—	10.9	4.9	—	—	—	—
121	Ear swab	21.9	18.5	13.9	—	20.5	18.5	16.5	—	24.5	14.9	13.9	—	—	7.9
175	Ear swab	13.9	9.5	—	—	9.5	7.9	—	—	9.5	—	—	—	—	—
133	Ear swab	16.5	12.5	7.5	—	14.9	12.5	—	—	15.5	11.9	—	—	9.9	13.5
<i>P. aeruginosa</i>															
Control strain	NCTC UK	26.5	20.5	10.9	—	23.9	19.9	13.9	—	21.5	20.5	12.5	—	9.5	12.5
104	Burn wound	16.5	13.9	9.5	—	12.9	7.5	5.9	—	10.9	7.9	—	—	—	—
58	Burn wound	18.5	12.5	8.9	—	11.5	8.5	5.9	—	13.5	9.9	5.9	—	—	12.5
108	Wound swab	12.5	10.5	9.5	—	10.5	6.9	—	—	7.5	—	—	—	—	—
603	Wound swab	6.5	—	—	—	6.5	—	—	—	3.9	—	—	—	10.5	15.9
573	Wound biopsy	9.5	7.5	—	—	9.5	—	—	—	5.5	—	—	—	10.9	13.9
880	Wound swab	10.5	7.9	—	—	8.9	5.9	—	—	9.5	5.9	—	—	9.5	12.5
591	Wound swab	9.5	6.9	—	—	8.5	—	—	—	6.5	4.9	—	—	8.5	10.5
337	Wound swab	15.5	10.5	9.9	—	10.9	8.5	4.9	—	10.5	5.9	—	—	—	—
305	Ear swab	9.5	5.5	—	—	6.5	—	—	—	6.5	—	—	—	6.5	10.9

#### Key

\* = undiluted honey sample

\*\* = gentamicin

a = 1:2 to 1:6 aq. dilutions of honey

b = zone of growth inhibition in mm indicating sensitivity of bacterial isolate

c = no zone of growth inhibition indicating bacterial resistance

## Results

Samples of honey from sources A, B, and C, as also gentamicin in 4.0 and 8.0 µg/ml dilutions, exhibited varying levels of antibacterial activity against the bacterial cultures tested as indicated by zones of growth inhibition (Table II). Undiluted honey from each source produced the strongest activity, followed by 1:2 and 1:4 dilutions in decreasing order. Only one isolate of *E. coli* - and none of *P. aeruginosa* - was inhibited by a 1:6 dilution. Percentage ranking shows that 100% of all the pseudomonal isolates, including the control strain (NCTC culture), were sensitive to every undiluted honey sample, followed respectively by 96.4%, 67.85%, and 64.28% sensitivity to 1:2 aq. dilution of honeys A, B, and C. Against *E. coli* isolates, 95.45% of all the cultures, including the control strain, were sensitive to every undiluted honey sample, with only one strain totally resistant. The 1:2 aq. dilution of honey A gave the same result but yielded respectively 86.36% and 77.27% with honeys B and C (Table III).

With gentamicin the 4.0 and 8.0 µg/ml tested recorded respectively 71.4% and 75.0% of sensitivity among *P. aeruginosa* isolates. Against *E. coli* the values were respectively 68.1% and 77.2%. Remarkably, one particular strain of *E. coli* (No. 126/108) that was sensitive to both 4.0 and 8.0 µg/ml of gentamicin was totally resistant to every undiluted honey and the aq. dilutions. Also, two isolates of *P. aeruginosa* from burns were inhibited by the three honey samples, compared with only one of them by gentamicin.

in burns<sup>14,15</sup> and other forms of trauma<sup>16</sup> has been recognized as evidence of chronic or acute infections.<sup>17</sup>

In a marked departure from the intuitive use of honey as an effective remedy,<sup>18</sup> various reports have associated the effectiveness of honey with its high antimicrobial activity,<sup>7,8</sup> which has been attributed to osmotic effect, acidity, hydrogen peroxide, phytochemical factors, and seven tetracycline derivatives.<sup>19,20</sup> The high antimicrobial activity found support in this study against the two Gram-negative bacteria tested along with the control strains. This was evident in the percentage levels of bacterial sensitivity - as high as 100% for *P. aeruginosa* and 96.4% for *E. coli*.

Remarkably, for the first time the strong activity of undiluted honey from each of the three sources of *P. aeruginosa* contrasted sharply with the strong activity of 1:2 aq. dilution of honey against *E. coli*.

Also of interest is the finding that the activity of gentamicin, both 4.0 and 8.0 µg/ml, was found to be virtually lower than that of undiluted honey or any of its aq. dilutions. This result supports earlier reports on honey and silver sulphadiazine in the treatment of burn wounds,<sup>9</sup> as also on honey and some antibiotics.<sup>10,11</sup> The variations recorded in the antibacterial activity of the types of honey tested were consistent with the reports of Jeddar<sup>8</sup> and Molan<sup>20</sup> and have been attributed to delayed levels of hydrogen peroxide/thermal stability of the glucose oxidase enzyme, non-peroxide factors, and the plant/floral source.<sup>21,22</sup> There is therefore a need for a microbiological assay of every honey sample in order to determine its "in-

**Table III** - Relative percentage sensitivity of *E. coli* and *P. aeruginosa* to honey and gentamicin

Isolate	Honey A				Honey B				Honey C				Gent. (µg/ml)	
	0*	1:2 <sup>a</sup>	1:4	1:6	0	1:2	1:4	1:6	0	1:2	1:4	1:6	4:0	8.0
E.C	95.5	95.5	72.7	4.5	95.5	86.4	59.1	0	95.5	77.3	36.4	0	68.2	77.2
P.A	100	96.4	57.1	0**	100	67.9	39.3	0	100	64.3	28.6	0	71.4	75.0

Key

0\* = undiluted honey

0\*\* = 0%, i.e. no sensitive isolate

a = 1:2 to 1:6 aq. dilutions of honey

E.C. = *Escherichia coli*

P.A. = *Pseudomonas aeruginosa*

## Discussion and conclusion

*P. aeruginosa* and *E. coli* are Gram-negative aerobic rods and can constitute environmental contaminants both of burn wounds and of other trauma, through dressing fluids or other sources, thereby causing sepsis. Specifically, the repeated occurrence of *Pseudomonas* spp. as pathogens

hibin number"<sup>21</sup> before it can be used as an antimicrobial agent. With appropriate standardization and with its lack of toxicity and allergy,<sup>23</sup> coupled with the encouraging results of this and other studies, the therapeutic application of honey in septic burn wounds and other forms of trauma could effectively complement standard antibiotics with beneficial healing effects.

**RÉSUMÉ.** La signification clinique de *Pseudomonas aeruginosa* et *Escherichia coli* constitue un facteur important pour la monitoring régulière de leur sensibilité vers les composés antimicrobiens utilisés depuis longtemps et aussi vers les composés nouveaux. Des isolés humains de ces organismes ont été collectionnés de diverses sources pathologiques et testés pour leur sensibilité à la gentamicine - un antibiotique aminoglycoside bien connu - et au miel, un produit naturel qui a eu un regain d'intérêt en considération de son application thérapeutique. Les Auteurs, utilisant une méthode de diffusion «agar-cup», ont trouvé que trois différents échantillons non dilués de miel et leurs dilutions aq. 1:2 à 1:6 exerçaient une activité sur 100% et 96,4% respectivement des isolés de *Pseudomonas aeruginosa* par rapport à 95,4% d'*Escherichia coli* utilisant les isolés non dilués ou les dilutions aq. 1:2 des échantillons du miel. L'activité de la gentamicine utilisée à concentrations de 8,0 et 4,0 µg/ml contre tous les deux organismes variait, mais généralement elle était inférieure à l'activité antibactérienne de chaque miel non dilué et à sa dilution aq. 1:2. Dans les cas d'insuccès thérapeutique avec la gentamicine ou avec d'autres antibiotiques similaires, le miel offre une alternative appropriée et meilleure dans la gestion des brûlures infectées et d'autres formes de lésions infectées, comme aussi dans la prophylaxie des lésions dues aux traumatismes.

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